EXHIBIT 8

THE COMPARATIVE PHARMACOLOGY OF THE ISOMERIC NITROGEN METHYL SUBSTITUTED HEPTYLAMINES

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Since the fundamental study of Barger and Dale (1910) of the relationship between the chemical constitution and physiological action of the sympathomimetic amines, numerous investigations have been made of many structurally and pharmacologically related compounds. Several of these investigations have been concerned with the influence on activity brought about by the substitution of a methyl group for one of the hydrogen atoms on the amino group of various beta-phenylalkylamines (Beyer, 1946) and a few with this change in cycloaliphatic amines (Swanson and Chen, 1948; Marsh, 1948a). No systematic study of this structural change in the field of simple aliphatic amines has been made, although one N-methyl aliphatic amine, 2-heptyl methylamine[1] or Oenethyl, is generally available.

In this study, the various nitrogen methyl heptylamines have been compared with the unsubstituted amines (see table 1)[2]. By limiting the problem to compounds with a total of six plus one carbon atoms in the primary structural unit, it is possible to have a wide range of activity and to determine any relationship between general spatial configuration and the influence of N-methylation without having to consider differences in molecular weight. N-methylation increases the molecular weight 12 per cent in this group of agents and this is probably less than the error of the most accurate procedure used in the investigation.

EXPERIMENTAL. Since repeated injections of the heptylamines yield diminished effects in animals (Marsh, 1948b), the following cross-over, indirect, method was used to avoid any possible error that could be introduced by such tachyphylaxis.

Nine mongrel dogs weighing 7.1–16 kgm. were given 20 mgm. of sodium thiopental per kgm. intravenously. A femoral artery and vein were quickly exposed; a hypodermic needle cannula attached to a Statham strain gage manometer and General Electric recording microammeter (Marsh, 1949) was introduced into the artery; one mgm. of scopolamine hydrobromide per kgm. was given; this was followed by 2, 4, 5, or 7 microgm. epinephrine

[1] This material is frequently referred to as "2-methylamino heptane." Since the naming of an organic compound possessing one primary functional group as a derivative of its parent hydrocarbon violates the fundamental nomenclature rules of the Commission on the Reform of the Nomenclature of Organic Chemistry of the International Union of Chemistry (cf. J. Am. Chem. Soc., **55:** 3905–25, 1933), we have referred to this compound by the name 2-heptyl methylamine.

[2] We are grateful to Dr. K. K. Chen and Mr. H. A. Shonle of the Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, Indiana for the sulfate salts of the unsubstituted amines (except 2-methyl-2-hexylamine). These were converted to the free base and dissolved in the theoretical amount of hydrochloric acid to make ten per cent solutions. We are grateful to Dr. R. O. Hauck of E. Bilhuber, Inc., Orange, N. J. for solutions of the hydrochlorides of the other amines.

per kgm. and then 0.7, 1.0, or 1.4 mgm. amine hydrochloride per kgm. The animal was allowed to recover and the procedure repeated at one-week intervals with different amines until at least four experiments had been carried out in all animals. The epinephrine equivalence was estimated to the nearest 0.5 microgm. and the geometric mean equivalence calculated (see table 1).

Sections of jejunum of eight white rabbits were suspended in Tyrode solution at 37°C., and aerated with 95 per cent oxygen-5 per cent carbon dioxide. After various preliminary concentrations were tried, one mgm. of amine hydrochloride per 50 ml. tissue bath was chosen as a standard concentration. After two minutes exposure to the drug, the bath was flushed out three to five times. The responsiveness of the segments to 0.5 microgm. epinephrine base was used as a control.

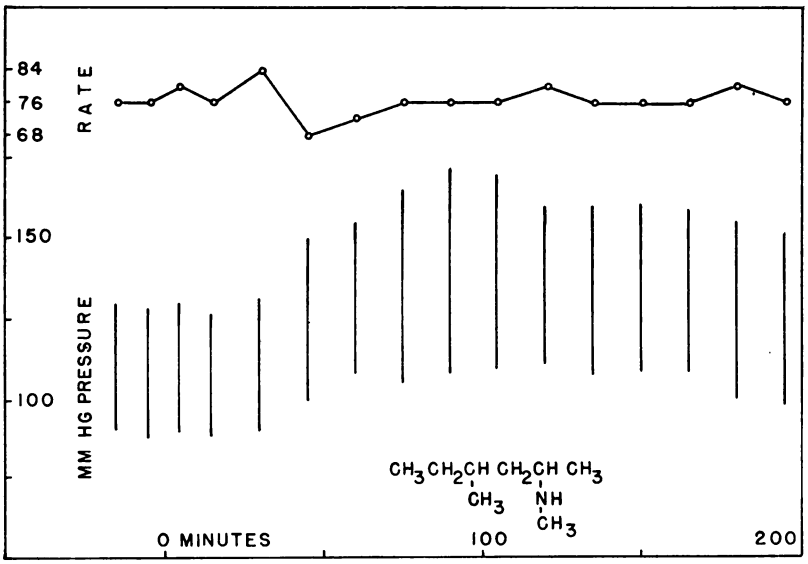

FIG. 1. Man (32 years old, 82 kgm.). Pulse rate above, systolic and diastolic blood pressure below measured by cuff sphygmomanometer. Three mgm. 4-methyl-2-hexyl methylamine hydrochloride per kgm. given orally in water at 0 time.

Mice were given the amine hydrochlorides intraperitoneally as 1 per cent solutions. Each mouse was kept in a separate pen at 22–23°C. Eight mice received each dose, and dose levels differed by 5 mgm./kgm. increments. All mice were observed four hours and the number dying in this time recorded. The data given in table 1 were all obtained from animals injected on a single day. The LD_{50}, to the nearest 5 mgm./kgm., was determined by the method of Litchfield and Wilcoxon (1949).

Five young adult males (22–32 years, 61–82 kgm.) were given 3 mgm. of the hydrochlorides of 4-methyl-2-hexyl methylamine, 4-methyl-2-hexylamine, 2-hexyl methylamine, and 2-hexylamine per kgm. orally with 200 ml. of water, four hours after a light morning meal. The agents were given at weekly intervals until all agents had been taken at least once. The systolic and diastolic blood pressure and the pulse rate were recorded every fifteen minutes for three hours while the subjects remained sitting quietly. Figure 1 is a typical plot of the data obtained.

RESULTS. The results of the animal experiments are summarized in table 1. Examination of the data indicates that replacement of one of the hydrogen

atoms on the amino nitrogen atom of the isomeric heptylamines does not markedly influence the vasopressor activity. Although some of the geometrical mean epinephrine equivalences are slightly greater or less for any pair of agents, the differences are not statistically significant. The responses of any given dog to the control doses of epinephrine varied as much as 18 per cent from week to week, although no dog had more than a 30 per cent differential variation in response to a given dose pair of epinephrine over the entire experimental period. In all four dogs in which the 4-methyl-2-hexyl and 2-heptyl pairs were crossed over, the 4-methyl-2-hexyl compounds were more active than the 2-heptyl type.

TABLE 1

NAME	PRESSOR ACTIVITY		RABBIT JEJUNUM—PERCENTAGE CHANGE IN AMPLITUDE PRODUCED BY 20 MGM./L.	MICE—I. P. LD_{50} MGM./KGM.
	Dose mgm./kgm. amine hydrochloride	Number of microgm. epinephrine/kgm. to have similar effect		
3-Heptylamine	1	0.5±	−20	90
3-Heptyl methylamine	1	0.5±	−50	70
2-Hexylamine	1	2.4	+100	60
2-Hexyl methylamine	1	2.1	+50	120
2-Methyl-2-hexylamine	1	1.0±	−50	85
	1.4	2.9		
2-Methyl-2-hexyl methylamine	1.4	3.0	−70	70
3-Methyl-2-hexylamine	1	2.0	−25	90
3-Methyl-2-hexyl methylamine	1	2.2	−60	70
4-Methyl-2-hexylamine	0.7	3.7	+40	185
	1	5.1		
4-Methyl-2-hexyl methylamine	1	4.6	−40	120
5-Methyl-2-hexylamine	1	3.2	−20	90
5-Methyl-2-hexyl methylamine	1	2.6	−70	65
2-Heptylamine	1	4.6	+70	95
2-Heptyl methylamine	1	4.2	+30	110

There was no obvious difference in duration of action between the methylated and unsubstituted amines.

Nitrogen methylation uniformly decreased the spasmogenic activity of a given compound if it produced an increase in amplitude of contraction of rabbit jejunum or further enhanced the relaxant effect if it produced a decrease in amplitude.

All the agents produce marked, irregular motor activity and convulsions in mice that received lethal doses. Pilomotor and exophthalmic responses are common. There is no obvious relationship between vasopressor effects in dogs, structure, presence or absence of a nitrogen methyl group, and lethal doses in mice.

About 45 minutes to one hour after the oral administration in man of 3 mgm. of one of the four more potent agents per kgm., the blood pressure begins to rise, the systolic-diastolic difference increases, and the pulse rate decreases. The

subjects complain of feeling hot or cold, that the skin tingles or itches, that there is a peculiar taste in the mouth or that the mouth is dry or the nose feels open. Pilomotor skin reactions are common. Mental confusion and inability to concentrate occasionally occur, although with no particular drug, and no evidence of central nervous stimulation as evidenced by excessive talkativeness is found. In four of the individuals the 4-methyl-2-hexyl methylamine is most potent in elevating the blood pressure, and in the other the 4-methyl-2-hexylamine is most active. The procedure was repeated to make certain no mistake had occurred in dosage or drug chosen. The results were the same within narrow limits. The individuals and the operator did not know which drug was given nor was the subject told the results of any measurements until after the experiment was completed.

Discussion. It seems fairly well established that nitrogen methyl substitution in the β-phenylalkylamine series decreases the vasopressor potency in dogs (Marsh, 1948a) as it does in the β-cyclohexylalkylamine series (Swanson and Chen, 1948; Marsh, 1948a) although it increases the activity of the β-cyclopentylalkylamines (Swanson and Chen, 1948). With this series of simple aliphatic amines, nitrogen methylation has little obvious effect on pressor potency. Regardless of the mechanism of action of these agents in producing this effect, apparently this chemical change influences neither absolute activity nor rate of disappearance. With oral doses in man of the potent members of this series, nitrogen methylation may actually influence the vasopressor response, and whether the nitrogen methyl substituted amine is more or less active than the unsubstituted amine may depend on the relative ability of the individual to absorb, transport, or eliminate (destroy or excrete) a particular type of agent.

The lack of relationship between structure and general toxicity or activity on isolated intestine is apparently a characteristic of the aliphatic amines, as it has been observed with related groups of agents (Marsh, 1948b; Marsh and Herring, 1950). However, other examples of nitrogen methylation enhancing the relative sympathomimetic depressant activity of amines on smooth muscle are known (Lands, 1949).

Although several publications on the activity of 2-heptyl methylamine have appeared (Ahlquist, 1944; Jackson, 1944; Roman-Vega and Adriani, 1946; Shaffer and Knoefel, 1950) and on the activity of 2-heptylamine and related compounds (Swanson and Chen, 1946; Marsh, 1948b) the various procedures have differed sufficiently that no comparison of the influence of nitrogen methylation is possible with the exception of the paper by Ahlquist who found 2-heptylamine to be more pressor and more toxic than 2-heptyl methylamine. None of the other isomeric heptyl methylamines seem to have been investigated. Both 2-heptylamine (Tuamine) and 4-methyl-2-hexylamine (Forthane) have been introduced as nasal vasoconstrictor agents (Chen, 1948) and 2-heptyl methylamine (Oenethyl) has been found to be a useful vasopressor substance for spinal anesthesia (Roman-Vega and Adriani, 1946). On the basis of the preliminary findings of our experiments, the 4-methyl-2-hexyl methylamine might prove equally useful for both of these purposes.

SUMMARY

1. The vasopressor activity of 3-heptyl, 2-methyl-2-hexyl, 3-methyl-2-hexyl, 4-methyl-2-hexyl, 5-methyl-2-hexyl, and 2-heptyl methylamine hydrochloride in dogs anesthetized with sodium thiopental and scopolamine has been determined and compared with the corresponding unsubstituted heptylamine hydrochloride and epinephrine. Nitrogen-methyl substitution does not significantly influence the vasopressor activity as compared with the unsubstituted amines and the compounds range from agents with very short duration of action and almost no pressor action to 4-methyl-2-hexyl methylamine which is about $\frac{1}{200}$ as active as epinephrine and has a long duration of action. The 2-heptyl methylamine is almost as active, and the corresponding unsubstituted amines may be slightly more active.

2. Nitrogen methylation of the isomeric heptylamines decreases the spasmogenic activity of the unsubstituted heptylamines on isolated rabbit jejunum or increases the relaxant activity.

3. All of the agents cause the death of white mice when given intraperitoneally in the dose range of 60–185 mgm./kgm. to individual mice kept at 22–23° C. and produce erection of hair, exophthalmos, increased motor activity and convulsions. Nitrogen methylation did not uniformly increase or decrease the toxicity of any given agent.

4. Oral doses of 3 mgm. of 4-methyl-2-hexyl methylamine, 4-methyl-2-hexylamine, 2-heptyl methylamine, and 2-heptylamine hydrochloride per kgm. produce marked vasopressor effects and have typical sympathomimetic actions. The 4-methyl-2-hexyl compounds were more active than the 2-heptyl derivatives.

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